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Chemical and microbiological quality of Anatolian Buffalo milk

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This study was carried out to determine the chemical and microbiological quality of Anatolian buffalo milk. A total of 120 Anatolian buffalo raw milk samples were collected monthly from Afyonkarahisar province, throughout the year for this study. In the chemical analysis of Anatolian buffalo milk samples the mean total solid value, non-solid fat, lipid, protein, lactose, ash and pH values were detected as 16.38%, 8.56%, 7.04%, 4.36%, 4.19%, 0.72% and 6.55, respectively. Total bacteria count (TCA), coliform, lactic acid bacteria (LAB), *Escherichia coli, Staphylococcus aureus* and yeast-mold (log_{10} cfu/ml) levels in the milk samples were detected as 6.36, 2.95, 5.74, 1.10, 2.46 and 2.63, respectively.

Key words: Anatolian buffalo, buffalo milk, chemical quality, microbiological quality

INTRODUCTION

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition in the world with its high nutritious property. If convenient storage temperatures are not paid attention, milk becomes a suitable propagation medium for microorganisms due to its biochemical composition and high water activity. Milk can be easily contaminated and spoiled when it is produced in unhygienic environment. Milk quality is directly related to its composition and hygiene (Oliver et al., 2005; Parekh and Subhash, 2008). Buffalo milk has become a research subject and received increasing attention in many countries due to its rich nutrient content (Amarjit and Toshihiko, 2003). Compared to cow milk, buffalo milk has a richer taste due to its contents of milk fat, protein, lactose, total dry matter, vitamin and minerals. These properties allow a wider variety for buffalo milk as raw material for milk products like cheese, butter and ice-cream (Fundaro et al., 2001). There has been an increasing demand for cheese made of buffalo milk in many countries throughout the world as it is an organic product (Bilal et al., 2006).

Turkish Statistical Institute (TUIK, 2011) stated that total amount of milk collected between January 2010 and July 2011 period was 4.309.006 tons, and buffalo milk accounted for 0.26% of total milk amount according to the data of Packed Milk and Milk Products Society (ASUD, 2010). Buffaloes are grouped into riverin and swap types. Buffaloes in Turkey originate from Mediterranean buffaloes, a sub-group of river buffaloes and they are named Anatolian buffaloes (Atasever and Erdem, 2008). Buffalo production does not have an important share due to its limited commercial use in Turkey; however, it is reported that global buffalo milk was 90 million tons in 2009 and accounted for 13% of total milk production. The same report stated that more than 90% of total buffalo milk in the word is produced by India and Pakistan. It is also reported that buffalo milk is produced in different countries including China, Egypt, Bulgaria, Italy and Iran

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(Spanghero and Susmel, 1996). Interest and investments in buffalo milk in different countries is increasing each year due to its unique taste and nutritious content (Amarjit and Toshihiko, 2003). It is reported that especially the products made of buffalo milk like mozzarella cheese, cream, ice-cream and yogurt have commercial importance, as well (Fundaro et al., 2001). As in raw milk, microorganisms could rapidly propagate in buffalo milk due to rich nutrient content. In previous studies carried out on microbiological properties of buffalo milk (Sethi et al., 1994; Sekerden et al., 1999; Han et al., 2007), coliform bacteria, *Escherichia coli*, lactic acid bacteria, yeast-mold and *Staphylococcus* spp., were isolated from buffalo milk. Saprophytic microorganisms in milk could spoil buffalo milk, while the presence of pathogen bacteria could pose a potential health threat (Boycheva et al., 2002; Han et al., 2007). Contamination of milk and milk products mostly results from human factor and unhygienic conditions. Milk is generally contaminated in milk collection places. Coliform bacteria are microorganisms in natural flora of human and animal intestinal tract and they are accepted as indicators for bacteriological quality of milk and milk products (Chatterjee et al., 2006). Furthermore, the presence of these microorganisms indicates the possible existence of enteric pathogens that could threat public health. The most important indicators for microbiological quality include total bacteria number, coliform, yeast and mold quantity and detection of specific pathogens and their toxins (Szita et al., 2008). Among all microorganisms, *E. coli* is an organism that frequently contaminates foods. They generally exist in milk and milk products due to inadequate sanitation (Jayarao and Henning, 2001). The main thing in microbiological investigation of milk is the determination of contamination degree and number of indicator microorganisms. Coliform bacteria are reported to define the suitability of milk for human consumption (Wells et al., 1991).

Besides microbiological quality of milk, its physical and chemical properties are also quite important. Changes in milk composition depend on many factors like genetic, lactation time, daily variations, birth, alimentation type, age, udder cleaning and season (Kilic and Kilic, 1994; Haenlein, 2003). These factors greatly affect the quality and processing ability of cheese, butter and other milk products (Lindmark-Manson et al., 2000; Barron et al., 2001; Lindmark-Manson et al., 2003). Geographic region, climate conditions and lactation period are known as seasonal changes and cited among factors affecting milk composition (Sethi et al., 1994; Suman et al., 1998; Sekerden et al., 1999; Boycheva et al., 2002; Waldner et al., 2002). Especially, there is an inverse proportion between ambient temperature and protein and fat content of milk. Solid fats tend to decrease with increasing air temperature. Fat, protein, casein and all fractions of nitrogen are affected by seasonal change (Ng-Kwai-Hang et al., 1984; Lacroix et al., 1996). It is reported that high ambient temperature causes clot hardness to decrease and clot formation rate and clotting time to increase (Sevi et al., 2001). At high ambient temperatures, fat concentration decreases, while lactation period extends (Kilic and Kilic, 1994; Sekerden, 1999). Some studies reported that significant changes occur in the amounts of short chain fatty acids, and they become minimum in winter and maximum in summer (Lindmark-Manson et al., 2003; Lock and Garnsworthy, 2003).

The main physical characteristics of milk are defined as pH and electrical conductivity; in addition, fat content of buffalo milk is the most variable milk component, which is caused by genetic and specific factors (Harmon, 1994; Hartet et al., 1999; Ma et al., 2000). Carbohydrates are preliminary form of glucose circulating as lactose in milk. Lactose level is reported to affect milk amount to be produced and the level of short chain fatty acids (Fernando et al., 1983; Hamman and Gyodi, 1994). High fat and calorie contents besides dry matter are regarded as the superior and distinctive property of buffalo milk (Soysal, 2006). Buffalo milk is processed into many products including butter, cream, hard and soft cheese, ice-cream and yogurt (Bilal et al., 2006).

The aim of this study carried out in Afyonkarahisar Province, located in an important region for buffalo milk and milk products with dense buffalo presence, was to determine the chemical and microbiological qualities of seasonally collected buffalo milk samples and to investigate the effect of seasonal change on milk composition.

MATERIALS AND METHODS

Milk samples

A total of 120 raw milk samples were collected every month from 10 small sized family enterprises (farms had ≤10 buffaloes) randomly selected in Afyonkarahisar Province, 10 samples from each, between September 2009 and August 2010. Two hundred and fifty milliliter (250 ml) of milk samples were taken from producer under aseptic conditions and transferred in sterile bottles to laboratory in cold chain 4°C' and then they were analyzed.

Chemical analyses

Fat, protein, lactose, total dry matter (DM) and ash contents of buffalo milk samples were determined by pre-calibrated LactoStar milk analysis device (FUNKE GERBER, Germany). pH values of milk samples were determined by InoLab (pH Level L 01280054) pH meter device.

Microbiological analyses

In the analysis, 10 ml of milk was taken from each milk sample and homogenized in sterile bags containing 90 ml of sterile buffered peptone water for 1-2 min and then dilutions were prepared by 10^8 and cultivations were performed. Cultivation was made on Plate Count Agar (Oxoid CM0325) using drop plate method in order to determine the number of mean total bacteria count (TCA) in the prepared dilutions (Anonymous, 2003). Violet Red Bile Agar (Oxoid-CM 107) was used to determine coliform bacteria count and incubated under aerobic conditions at 37°C for 24 h (Anonymous, 1993). Cultivation was made on MRS Agar (Oxoid-CM 0361) for

Table 1. Chemical analysis results of Anatolian Buffalo milk.

Season	Total solids (%w/w)	Non fat solid (%w/w)	Fat (%v/v)	Protein (%w/w)	Lactose (%w/w)	Ash (%w/w)	pH
Winter (n=30)	17.41±0.54[a]	8.89±0.28[a]	7.67±0.55[a]	4.90±0.26[a]	3.99±0.12[b]	0.78±0.07[a]	6.68±0.11[a]
Spring (n=30)	15.61±0.74[c]	8.49±0.23[b]	6.38±0.66[b]	4.03±0.16[c]	4.45±0.20[a]	0.69±0.07[b]	6.45±0.06[c]
Summer (n=30)	15.90±1.03[c]	8.49±0.27[b]	6.70±0.89[b]	4.05±0.14[c]	4.43±0.25[a]	0.66±0.06[b]	6.45±0.12[c]
Autumn (n=30)	16.59±0.57[b]	8.37±0.25[b]	7.40±0.51[a]	4.46±0.18[b]	3.91±0.21[b]	0.77±0.07[a]	6.55±0.12[b]
Mean (120)	16.38±1.01	8.56±0.32	7.04±0.84	4.36±0.40	4.19±0.32	0.72±0.08	6.55±0.13

a,b,c; The difference between mean values with different letter in the same column is statistically significant P < 0.01.

Table 2. Microbiologic analysis results of Anatolian Buffalo milk (log10 cfu/ml).

Season	TBC	Coliform	LAB	*E. coli*	*S. aureus*	Yeast-Mold
Winter (n=30)	6.26±0.37[b]	2.83±0.22[c]	5.65±0.26[b]	0.98±0.13[b]	2.32±0.23[c]	2.50±0.32[c]
Spring (n=30)	6.33±0.19[b]	2.90±0.18[bc]	5.77±0.21[ab]	1.15±0.21[a]	2.43±0.21[bc]	2.63±0.22[b]
Summer (n=30)	6.52±0.23[a]	3.11±0.14[a]	5.89±0.41[a]	1.18±0.15[a]	2.62±0.22[a]	2.78±0.20[a]
Autumn (n=30)	6.35±0.22[b]	2.95±0.17 [a b]	5.67±0.28[b]	0.92±0.14[b]	2.47±0.18[b]	2.64±0.19[b]
Mean (120)	6.36±0.28	2.95±0.21	5.74±0.31	1.10±0.17	2.46±0.24	2.63±0.25

[a,b,c]; The difference between mean values with different letter in the same column is statistically significant P< 0.01
TBC: Total Bacteria Count LAB: Lactic Acid Bacteria

lactic acid bacteria (LAB) count and left for 48-72 h of incubation under anaerobic conditions at 30°C (Gas Generation Kit Oxoid BR 0038) (Harrigan and MacCance, 1976).

Cultivations were made on Tryptone Bile X- Glucuronide Medium (TBX) (Oxoid-CM945) for *E. coli* count and incubated at 44°C for 18-24 h (Anonymous, 2001). Egg Yolk Tellurite emulsion (Oxoid-SR54) was included for *S. aureus* count and performed in Baird Parker Agar (Oxoid -CM275) at 37°C for 24-48 h aerobic conditions (Anonymous, 1999). Cultivations were made on Rose Bengal Chloramphenicol Agar (Oxoid-CM549) for yeast and mold count and incubated at 25°C for 72 h under aerobic conditions (Anonymous, 1987).

Statistical analyses

The microorganism numbers detected in this study were transferred to base 10 logarithm values and then statistical data was obtained by SPSS statistics software. One way variance analysis was performed to determine the differences between microorganism numbers and chemical parameters in terms of seasons, and Duncan test was applied to determine differences among the means.

RESULTS

Chemical analysis

As a result of the chemical analyses of milk samples collected from buffaloes in different seasons, the total solids (TS) (16.38% ± 1.01), non-fat solids (NFS) (8.56 ± 0.32), fat (7.04% ± 0.84), protein (4.36% ± 0.40), lactose (4.19% ± 032), ash (0.72% ± 0.08) and pH (6.55 ± 013) values were determined (Table 1). Generally milk yield was determined to increase in plains, humid and rainy areas, while fat content was found higher in cold and mountainous areas. The data of this study indicated that milk fat and protein contents increase concurrently, while TS, NFS, fat, protein, ash and pH values are highest in winter, while TS, fat and protein levels are lowest in spring.

Microbiological analysis

Regardless of seasons, the mean total bacteria count (TBC), coliform bacteria, lactic acid bacteria (LAB), *E. coli*, *S. aureus* and yeast-mold values (log_{10} cfu/ml) were determined as 6.36 ± 0.28, 2.95 ± 0.21, 5.74 ± 0.31, 1.10 ± 0.17, 2.46 ± 024 and 2.63 ± 0.25, respectively (Table 2).

Contamination levels of milk with TBC, coliform, *S. aureus* and yeast-mold were found lower in winter than in other seasons, while the lowest *E. coli* contamination was detected in autumn. The highest contamination levels with these bacteria were observed in summer. Microbiological values of milk samples were found at similar levels in spring and autumn.

DISCUSSION

In this study, the mean total solids, non-fat solids, fat, protein, lactose and pH levels of milk samples collected in different seasons were determined as 16.38%, 8.56%, 7.04%, 4.36%, 4.19%, 0.72% and 6.55, respectively. Fat, protein and ash contents were determined to decrease in hot summer months, while lactose content was reported to increase (Yöney, 1974). It was reported that buffalo milk contain has higher nutritious values with higher pro-

protein, fat, lactose and TS than cow milk (Fundora et al., 2001; Lidmark-Manson et al., 2003). Different researchers reported that alimentation, lactation period, milking frequency, milking method and season have important effects on physicochemical parameters of buffalo milk (Sethi et al., 1994; Suman et al., 1998; Sekerden et al., 1999; Boycheva et al., 2002; Waldner et al., 2002).

In this study, the mean lactose level of buffalo milks (4.19% ± 032) was found lower than those reported by Han et al. (2007) and Najdenova and Dimitrov (2003). On the other hand, milk fat is the most changeable milk component. Fat content is affected by many factors. The most important factors are seasonal change and lactation period. Fat, protein and ash contents tend to increase in winter, and milk yield is reported to increase in the later periods of lactation, while fat and protein contents decrease. It is also reported that habitat and feeding pattern are quite effective on milk fat and protein levels, and milk protein and NFS contents of animals grazing in summer are higher than those of animals closed-fed in winter (Yöney, 1974; Sevi et al., 2004). In this study, the mean fat (7.04 ± 0.84 %v/v), total solids (16.38 ± 1.01% w/w) and protein (4.36 ± 0.40% w/w) contents are slightly lower than those reported by Najdenova and Dimitrov (2003) and Han et al. (2007). Similar results were reported by Sarfarz et al. (2008) and Çelik et al. (2001), but the values of the present study were higher than found by Kanwal et al. (2004). On the other hand, Ariota et al. (2007) reported fat and protein contents of buffalo milk as 8.71 and 3.86%, respectively, and pH as 6.58.

The mean ash values (0.72 ± 0.08% w/w) of Anatolian buffalo milk are similar to the values reported by Celik et al. (2001) and Sarfarz et al. (2008), but higher than the value found by Sekerden and Avsar (2008). The mean pH (6.55 ± 0.13) of Anatolian buffalo milk is similar to the values reported by Han et al. (2007) and Sekerden and Avsar (2008), but higher than those of Aurelia et al. (2009).

Variance analysis was used to investigate chemical composition. Total solids, protein, non- fat solids and pH values were significantly higher ($p<0.01$) in winter than in other seasons. On the other hand, the lactose content was found higher ($p<0.01$) in spring and summer than in winter and autumn. The mean fat and ash contents were found highest in winter and autumn.

Similar to milk components, microbiological quality of milk changes by ambient temperature. In this study, the mean total bacteria count (TBC), coliforms, lactic acid bacteria, *E. coli*, *S. aureus* and yeast-mold (log_{10} cfu/ml) levels were determined as 6.36±0.28, 2.95±0.21, 5.74 ± 0.31, 1.10 ± 0.17, 2.46 ± 024 and 2.63 ± 0.25, respectively, and microorganism load was determined to increase in warm months.

The Turkish National Standard (TSE 1018) for TBC was 1.0 x 10^5 cfu/ml for raw cow milk. The EU specification (EU Directive 92/46/EEC, 2004) for raw buffalo milk is an average of 5 x 10^5cfu/ml TC. In this study, the mean TBC was determined as 2.30x10^6 cfu/ml. This level is higher than both the TSE and EU standards. The main reason for these relatively higher counts of TBC should be ascribed to poor hygiene conditions during milking, collection and transport.

S. aureus may access bulk milk either by direct excretion from the udder with clinical and subclinical staphylococcal mastitis, or by fecal contamination (Callon et al., 2008). Interchange of staphylococcal strains and poor microbiological quality of raw milk may be attributed to skin particles in the environment and poor sanitary practice (Normanno et al., 2007).

In another study carried out in China, TBC, LAB, yeast-mold, coliforms, *E. coli* and *S. aureus* (log $_{10}$ cfu/ml) levels in 120 buffalo milk samples were determined as 5.59 4.62, 1.79, 2.42, 1.53 and 1.68, respectively (Han et al., 2007). Coroian et al. (2010) reported mean coliform bacteria, yeast-mold and aerobe mesophile general creature levels in 42 Romanian buffalo milk samples as 4.96 ± 0.45/ml, 633.47 ± 0.01/g and 4.46 ± 0.11 x 10^5/ml, respectively and they also determined 3.27 log cfu/ml of *E. coli* in three samples. According to the results of this study, coliform, *E. coli* and yeast-mold levels were lower than those reported by Coroian et al. (2010); however, TBC level was found higher. The same researchers carried out a study on Murrah buffalo species and determined coliform, *E. coli, S. aureus* and yeast-mold levels (log_{10} cfu/ml) as 3.95 ± 0.07, 1.80 ± 0.23, 1.80 ± 0.23 and 1.33 ± 0.46, respectively. Accordingly, coliform and *E. coli* levels were lower than those determined in the present study, while *S. aureus* and yeast- mold levels were higher. Similarly, Desmasures et al. (1997) studied cow milk in different seasons, and reported that TBC, LAB, yeast, coliform and *S. aureus* levels (log_{10} kob/ml) were 7.1x10^3, 1.8x10^2, 7.2x10^1, 5.7x10^1 and 4.5x10^2, respectively, in winter, while these levels were determined as 8.6x10^3, 1.8x10^2, 8.4x10^1, 7.7x10^1 and 3.5x10^2 in summer; accordingly, the levels determined in the present study in winter period were lower than those reported by Desmasures et al. (1997). Considering summer levels, TBC was found high, while others were low. Similarly, Ali et al. (2010) determined mean TBC, LAB, coliform, *E. coli* and *S. aureus* levels in cow milk as 5.86 ± 0.31, 4.47 ± 0.44, 2.76 ± 0.18, 1.63 ± 0.20 and 1.92 ± 0.47, respectively.

In another study on raw buffalo milk samples, TBC, *E. coli*, and yeast levels (log_{10} cfu/ml) were determined between 3.4 x 10^5-4.0 x 10^7, 2.0x10^1-1.7 x 10^4 and 2.7 x 10^2-1.7 x 10^4, respectively (Braun and Preuss, 2007) compared with this study. LAB constituted a major part of the microflora with an average of 5.74±0.31 log cfu/ml (Han et al., 2007) and mean LAB level in buffalo milk was reported as 4.62 ± 0.12 log cfu/ml (Lingathurai et al., 2009) and 4.46 ± 0.44 cfu/ml, while it was found 4.47 ± 0.44 cfu/ml in raw cow milk (Ali et al., 2010). Boycheva et al. (2002) observed that LAB was predominant in Bulgarian buffalo milk. High level of LAB in raw milk would result in undesired fermentative acidity, and it is suggested to take effective precautions for preventing this kind of spoilage (Han et al., 2007).

Chemical composition of buffalo milk provides perfect opportunities for the development of local milk industry and providing nutrient element needs of humans. In addition, the presence of pathogens, indicators and index microorganisms in raw milk and products made of inadequately heat-treated milk could pose a threat for public health.

Livestock enterprises making milk production are composed of family enterprises in villages and towns with large numbers. Small sized production units have difficulty in obtaining inputs and services like adequate shelter, feed, technical information, veterinary services for buffalo and cow dairy production. It is possible to precisely organize hygienic and technological stages from production to consumption of milk and milk products only when all potentials are combined. In this regard, the principal that quality milk comes from healthy udder, healthy animal and clean environment gains great importance.

The important rule in food processing is the good quality of raw material. A good quality of end product cannot be obtained from a raw material with poor hygienic quality. Spoilage process of milk starts with milking. The previous studies confirmed that milk hygiene does not receive enough attention in Turkey. Considering the unhealthy conditions in milk production and other contamination sources in milk processes, milk can be a conveyor of pathogens threatening public health. Microorganisms cause rapid souring, spoilage and undesired color, taste and bitterness in milk and thus resulting in poor quality. It is reported that many epidemic disorders of milk origin are caused by dirty hands of workers in milk production, dirty tools and equipment, insects and dirty water sources. Provision of microbiological quality parameters of raw milk and milk products plays an important role in quality control. It is necessary to minimize technological and economic losses in milk processing and obtain a longer shelf life.

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